AD-A052 299 MIAMI UNIV FLA LEO RANE RESEARCH LAB F/G 6/15
CHEMOTHERAPY OF 'TRYPANOSOMA RHODESIENSE'.(U)
MAY 74 D S RANE DADA17-72-C-2142
UNCLASSIFIED NL

END
DATE FILMED
5-78
DDC

(12)
B.S.

REPORT NUMBER TWO

CHEMOTHERAPY OF TRYPANOSOMA RHODESIENSE

ANNUAL SUMMARY REPORT

DORA S. RANE

For the period of June 1, 1973 to May 31, 1974

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D. C. 20314

Contract No. DADA 17-72-C-2142

Dr. Leo Rane Research Laboratory
University of Miami
Miami, Florida 33142

DDC AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Two	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER: (9) Rept. no. 2 (Annual) DL
4. TITLE *(and Subtitle)*: (6) CHEMOTHERAPY OF TRYPANOSOMA RHODESIENSE.	5. TYPE OF REPORT & PERIOD COVERED: Annual Report, June 1, 1973-May 31, 1974 (1 Jun 73 - 31 May 74)
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): (10) Dora S. Rane	8. CONTRACT OR GRANT NUMBER(s): (15) DADA 17-72-C-2142
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Dr. Leo Rane Research Laboratory, University of Miami, Miami, FL 33142	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: (11) 31 May 74
11. CONTROLLING OFFICE NAME AND ADDRESS: U. S. Army Medical Research & Development Command, Washington, D. C. 20314	12. REPORT DATE: May 31, 1974
	13. NUMBER OF PAGES: 7
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*	15. SECURITY CLASS. *(of this report)*: (12) 9p.
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

T. rhodesiense blood-induced infection in mice - based on mortality.

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Primary screen-quantitative evaluation of potential trypanosomicidal activity.

409 663

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

Foreword

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

The test system described herein was developed specifically to evaluate the trypanosomicidal activity of large numbers of candidate compounds.* Based on blood-induced Trypanosoma rhodesiense infections in mice, it performs as a primary screen or as a secondary screen and confirmatory test and gives precise quantitative evaluations of chemical compounds that demonstrate potentially useful therapeutic and/or prophylactic activity in T. rhodesiense infections. Consequently, it is also a helpful guideline in the synthesis of new active agents.

These agents include: (1) chemicals structurally related to compounds of known value in the treatment or prevention of T. rhodesiense infections; (2) chemicals structurally unrelated to compounds of known value in the treatment or prevention of T. rhodesiense infections and; (3) structural analogues of compounds that have demonstrated activity in our screening procedure and represent novel chemical groups.

All candidate compounds were obtained from the Department of Medicinal Chemistry at the Walter Reed Institute of Research.

Table I summarizes the number of compounds tested and the number of mice used from June 1, 1973 through May 31, 1974.

*Designed, standardized and put into operation by the late Dr. Leo Rane.

TABLE I

MONTHLY SCREENING LEVELS

JUNE 1, 1973 - MAY 31, 1974

MONTH	NO. COMPOUNDS	NO. MICE
JUNE	57	1210
JULY	54	1210
AUGUST	106	2390
SEPTEMBER	224	3520
OCTOBER	151	2385
NOVEMBER	152	2460
DECEMBER	76	1200
JANUARY	154	2390
FEBRUARY	175	2705
MARCH	151	2345
APRIL	205	2345
MAY	76	1200
TOTALS	1581	25360

Our own colony of ICR/HA Swiss mice provided all the test animals needed in this operation. Using mice of a given age, sex and weight and a standard inoculum of the Wellcome CT-strain of T. rhodesiense, it has been possible to produce a consistently uniform disease fatal to 100 percent of untreated animals within 4-6 days.

Test compounds were administered either parenterally or orally in a single dose on the day of infection.

Activity was determined by responses to candidate compounds by T. rhodesiense infections in mice as expressed in comparisons of the maximum survival time of the treated trypanosomiasis-infected animals and the survival time of the untreated trypanosomiasis-infected controls. To be classified as active, a compound must suppress the disease and produce an increase of at least 100 percent in the life span of the treated animals over that of the untreated controls.

Acceptance of a test compound's activity was also predicated on the margin between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED). A maximum tolerated dose is defined as the highest dose causing no more than one of five animals to die and a minimum effective dose as the minimum dose increasing the life-span of treated animals 100 percent over the life span of untreated controls.

METHODS

Animal Hosts. Our own breeding colony of ICR/HA Swiss mice has supplied all the animals used in this screening procedure. Test

animals weigh 30-32 grams, weight variations in any given experimental or control group being carefully limited to 3 grams. In any given test all animals have been of a single sex and approximately of the same age.

Animals on test are housed in metal topped plastic cages, fed a standard laboratory diet and given water ad lib.

They are kept in a room maintained at 84°F (± 2°F) and a relative humidity of 66% (± 2%).

Test Procedure. Test animals receive an intraperitoneal injection of 0.5 cc of a 1:50,000 dilution of heparinized heart's blood drawn from donor mice infected 3 days earlier.

The donor line is maintained by 3-day blood passes, each animal receiving 0.1 cc of a 1:500 dilution of heparinized heart's blood drawn from a 3-day donor. Donors, like test animals, weigh 30-32 grams, weight variations for each pass being limited to 3 grams.

To check factors such as changes in the infectivity of our T. rhodesiense strain or in the susceptibility of the host or to detect technical errors, one group of infected animals treated with stilbamidine isothionate and a second group of infected animals treated with 2-hydroxy-stilbamidine isothionate at dose levels known to produce definitive increases in survival time are included as positive controls in every experiment.

Drug Administration. Test compounds are dissolved or suspended in peanut oil and prepared in three or more graded doses. At least three different doses of each test compound are included in an experiment.

Treatment consists of a single dose administered subcutaneously or orally on the day of infection. Deaths that occur before the fourth day, when untreated controls begin to die, are regarded as the result of a compound's toxic effect and not as the result of action by the infecting parasite.

Increases in the dose levels of highly active compounds usually are followed by increases in the survival time of the treated mice. However, if an active drug is toxic for the host, the toxicity of this compound may become a limiting factor to changes in dose levels.

Treated animals alive at the end of 30 days are considered as cured. Blood and tissues of cured animals subinoculated into fresh hosts have given no evidence of the disease.

Drug Activity. An increase of 100% in survival time is considered the minimum significantly effective response for a candidate compound. Clearly inactive compounds are rejected after one test, borderline compounds after two tests.

Active compounds are subjected to a dose-response curve so that the spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) may be established.

COMPOUNDS WITH DEFINITE CHEMOTHERAPEUTIC ACTIVITY AGAINST *TRYPANOSOMA RHODESIENSE* INFECTIONS IN MICE

During the opening period of this project, June 1, 1972 - May 31, 1973, our screening procedure was developed and its reliability established. 2,752 selected compounds were screened,

including a number of agents known to be effective in Trypanosoma rhodesiense infections and drugs drawn from our antimalarial program. Of these, 68 demonstrated a degree of activity sufficient to produce at least 100 percent increases in the survival time of treated T. rhodesiense infected mice.

1,581 compounds were tested in the period, June 1, 1973 - May 31, 1974, and of the 185 that demonstrated a degree of activity producing an increase of at least 100 percent in the survival time of treated T. rhodesiense infected mice, 92 were administered subcutaneously and 93 orally.

This breakdown is significant since: (1) activity evaluations provided in our screening procedure are precise and quantitative; (2) dose response curves of active compounds administered subcutaneously show a wider spread between the maximum tolerated dose (MTD) and the minimum dose producing a significant effect (MED) than dose response curves of active compounds administered orally and; (3) these dose responses also reveal a wider spread of toxic effects when active compounds toxic for the host are administered subcutaneously rather than orally.

DISTRIBUTION LIST

4 copies	HQDA (SGRD-RP) WASH DC 20314
12 copies	Defense Documentation Center (DDC) ATTN: DDC-TCA Cameron Station Alexandria, Virginia 22314
1 copy	Superintendent Academy of Health Sciences, US Army ATTN: AHS-COM Fort Sam Houston, Texas 78234
1 copy	Dean School of Medicine Uniformed Services, University of the Health Sciences Office of the Secretary of Defense 6917 Arlington Road Bethesda, MD 20014